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Abstract: INTRODUCTION: Intestinal inflammation alters colonic afferent nerve sensitivity which may contribute to patients' perception of abdominal discomfort. We aimed to explore whether mast cells and the cyclooxygenase pathway are involved in altered afferent nerve sensitivity during colitis. METHODS: C57Bl6 mice received 3% dextran-sulfate sodium (DSS) in drinking water for 7 days to induce colitis. Control animals received regular water. On day 8 inflammation was assessed in the proximal colon by morphology and histology. Extracellular afferent nerve discharge was recorded from the mesenteric nerve of a 2 cm colonic segment. Subgroups were treated in vitro with the mast cell stabilizer doxantrazole (10 M) or the cyclooxygenase inhibitor naproxen (10 M). RESULTS: DSS colitis resulted in morphological and histological signs of inflammation. At baseline, peak firing was 11 ± 2 imp s⁻¹ in colitis segments and 5 ± 1 imp s⁻¹ in uninflamed control segments ($p < 0.05$; mean \pm SEM; each $n=6$). In colitis segments, afferent nerve discharge to bradykinin (0.5 M) was increased to 47 ± 7 compared to 23 ± 6 imp s⁻¹ in recordings from non-inflamed control tissue ($p < 0.05$). Mechanosensitivity during luminal ramp distension (0-80 cm H₂O) was increased reaching 24 ± 5 imp s⁻¹ at 80 cm H₂O during colitis compared to 14 ± 2 in non-inflamed controls ($p < 0.05$). Doxantrazole or naproxen reduced afferent discharge to bradykinin and luminal ramp distension in colitis segments to control levels. CONCLUSION: Intestinal inflammation sensitizes mesenteric afferent nerve fibers to bradykinin and mechanical stimuli. The underlying mechanism responsible for this sensitization seems to involve mast cells and prostaglandins.

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Mast cells and the cyclooxygenase pathway mediate colonic afferent nerve sensitization in a murine colitis model.

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Abstract

Introduction: Intestinal inflammation alters colonic afferent nerve sensitivity which may contribute to patients' perception of abdominal discomfort. We aimed to explore whether mast cells and the cyclooxygenase pathway are involved in altered afferent nerve sensitivity during colitis.

Methods: C57Bl6 mice received 3 % dextran-sulfate sodium (DSS) in drinking water for 7 days to induce colitis. Control animals received regular water. On day 8 inflammation was assessed in the proximal colon by morphology and histology. Extracellular afferent nerve discharge was recorded from the mesenteric nerve of a 2 cm colonic segment. Subgroups were treated *in vitro* with the mast cell stabilizer doxantrazole (10^{-4} M) or the cyclooxygenase inhibitor naproxen (10^{-5} M).

Results: DSS colitis resulted in morphological and histological signs of inflammation. At baseline, peak firing was 11 ± 2 impsec $^{-1}$ in colitis segments and 5 ± 1 impsec $^{-1}$ in uninflamed control segments ($p < 0.05$; mean \pm SEM; each $n = 6$). In colitis segments, afferent nerve discharge to bradykinin ($0.5 \mu\text{M}$) was increased to 47 ± 7 compared to 23 ± 6 imp sec $^{-1}$ in recordings from non-inflamed control tissue ($p < 0.05$). Mechanosensitivity during luminal ramp distension (0 - 80 cmH $_2$ O) was increased reaching 24 ± 5 impsec $^{-1}$ at 80 cmH $_2$ O during colitis compared to 14 ± 2 in non-inflamed controls ($p < 0.05$). Doxantrazole or naproxen reduced afferent discharge to bradykinin and luminal ramp distension in colitis segments to control levels.

1 *Conclusion:* Intestinal inflammation sensitizes mesenteric afferent nerve fibers
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3 to bradykinin and mechanical stimuli. The underlying mechanism responsible
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5 for this sensitization seems to involve mast cells and prostaglandins.
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10
11 **Keywords:** afferent nerve, colitis, cyclooxygenase, mast cell, visceral
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13 sensitivity
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Introduction

Chronic inflammatory bowel disease (IBD) is characterized by abdominal pain and discomfort for affected patients. These symptoms are initiated by the release of an array of inflammatory mediators with subsequent sensitization of visceral afferent nerves (1). Despite this common knowledge, our understanding of mechanisms that may contribute to this sensitization and subsequent perception of pain during intestinal inflammation is limited.

Visceral sensitivity during intestinal inflammation is most commonly studied in rat or mice by characterization of the visceromotor response i.e. behaviour and/or muscle contraction of the abdominal wall during intestinal distension (2). This method is indirect and the visceromotor response as outcome parameter for visceral sensitivity may be modulated at various levels such as the intestinal wall, afferent pathways, the central nervous system (CNS), and efferent pathways (3, 4). Contrary to this indirect and rather unspecific method of studying visceral sensitivity, models of direct recordings from extrinsic afferent nerves innervating the intestine allow to measure peripheral afferent sensitivity directly without potential modulation in the CNS. These direct recordings also allow to test specific chemical or mechanical stimuli and the effect of antagonists interfering with the action of different mediators. Thus, direct recordings from afferent nerves permit to study the potential involvement of different mechanisms specifically (5).

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2
3 Coldwell et al. characterized afferent nerve responses to the mediator
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6 5-hydroxytryptamine (5-HT) and localized mechanical stimulation of the colon
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8
9 to project in the corresponding lumbar splanchnic nerve (6). They found
10
11 increased sensitivity to both stimuli during acute dextran sulphate sodium
12
13 (DSS) induced colitis which persisted for 5-HT after the acute inflammation
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15
16 had subsided. Further studies provided evidence that transient receptor
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19 potentials of the vanilloid type 1 (TRPV1, 7, 8), type 4 (TRPV4, 9), the transient
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21
22 receptor potential channel A1 (10), and protease activated receptor PAR₂ (9)
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25 on colonic afferent nerve terminals are involved in afferent sensitization during
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28 experimental colitis.
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34 In the present study, our interest was to investigate the role of mast cells and
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36 the cyclooxygenase pathway for afferent sensitivity during colitis. We
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39 specifically investigated afferent sensitivity to bradykinin (BK) as its release
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41
42 and sensitizing action on afferent nerve terminals is a crucial mechanism
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45 leading to the generation of pain in somatic models (11). For the same reason
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48 the afferent nerve response to mechanical distension was also tested.
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53 We hypothesized that BK- and mechano-sensitivity of afferent nerve fibers
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56 innervating the colon is increased during DSS induced colitis, and that mast
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59 cells and the cyclooxygenase pathway are involved.
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Methods

Animals / DSS colitis

Experiments were performed with male C57BL/6 mice (Charles River, Sulzfeld, Germany) weighing approximately 20g. Animals were held under a 12h/12h dark/light cycle with free access to food and water before and during experiments. Animal experiments were approved by the local Institutional Review Board (Regierung von Oberbayern). Colitis was induced by feeding animals 3 % dextran-sulfate sodium (DSS) which was dissolved in the animals' drinking water and given for seven consecutive days as described previously (12, 13). Control animals received vehicle only i.e. normal tap water. Animals were sacrificed on day eight after the beginning of DSS or vehicle administration by inhalation of an anesthetic overdose (Isoflurane, Abbott, Baar, Switzerland). Then, after a quick laparotomy, the colon was removed with the adjacent mesentery attached.

Tissue preparation for afferent nerve recordings

The colon and its mesentery were placed in a culture dish containing ice-cold Kreb's solution and the cecum was cut off. Then, under stereoscopic vision (operating microscope, Wild M3Z, Heerburg, Switzerland), the mesentery was dissected off the colon from distal to proximal except for the first 2 cm of proximal colon i.e. the first part distal to the cecum. These 2 cm of ascending

colon with the mesentery attached were prepared for afferent nerve

[recordingsrecordings.](#)

Technique of afferent nerve recordings

The colonic segment was placed into a custom-made organ bath that consisted of two chambers. In a perfusion chamber, the segment was superfused with Kreb's buffer gassed with a O₂/CO₂ mixture (95 % / 5 %; composition of Kreb's (mM): Na⁺ 143.5, K⁺ 5.9, Cl⁻ 126, Ca²⁺ 2.5, Mg²⁺ 1.2, H₂PO₄ 1.2, SO₄ 1.2, HCO₃⁻ 25, glucose 10 and sodium butyrate 1, pH 7, superfused at a rate of 10 ml min⁻¹, temperature 32°C). In order to eliminate spontaneous colonic motility that rendered afferent nerve recordings impossible in pilot experiments, the L-type calcium channel blocker nifedipine was added to the perfusion solution at a concentration of 1 µM as described by others (14). Continuous superfusion with Kreb's in the perfusion chamber was ensured with the help of a pump (Minipuls 3, Gilson, France).

The mesenteric arcade next to the colonic segment was guided through an opening into a recording chamber. The opening was sealed with Vaseline before the recording chamber was filled with colourless heavy liquid paraffin (32 Celsius) for insulation. Both ends of the colonic segment were cannulated and tied. The lumen was continuously perfused with Kreb's from the proximal side of the colon (10 ml per hour), while the distal cannula remained open to

atmosphere during the experiment unless mechanical ramp distension was performed (see below). Intraluminal pressure was monitored continuously by a separate channel in the proximal cannula which was connected to a pressure transducer (Neurolog pressure amplifier NL 108, Digitimer Ltd., Welwyn Garden City, UK). The pressure at baseline was typically 0.5 - 2 cmH₂O.

In the recording chamber, the mesenteric nerve bundle was dissected out of the mesentery attached to the colonic segment. The mesenteric nerve was then attached to one arm of a bipolar platinum recording electrode, with a fiber of connective tissue wrapped around the other electrode serving as a reference. The electrodes were connected to a single channel 1902 preamplifier/filter (Cambridge Electronic Design (CED), Cambridge, UK), and the signal was amplified 10.000 times and filtered with a bandwidth of 100 Hz to 1 kHz. Signals from the pressure transducer recording the intraluminal intestinal pressure were relayed to another single channel 1902 preamplifier/filter. The output from the preamplifier/filter and the signals from the pressure transducers were transferred to a power Micro 1401 interface system (CED) saved and viewed online by running Spike 2 software (version 4.01; CED).

Experimental protocol

Once a stable recording from the mesenteric colonic nerve was established for 15 minutes, spontaneous afferent nerve discharge was recorded at baseline for 10 minutes. Then, mechanical stimulation of the colonic segment was performed by ramp distension to 80 cmH₂O in approximately 120 seconds. For this aim, the outlet of the cannula in the lumen of the intestinal segment was clamped and perfusion with Kreb's solution was continued (10 ml per hour). Thereafter, the distal outlet was reopened and a minimum of 10 minutes was allowed for afferent discharge to return to baseline levels before the perfusion was stopped and BK was administered into the organ bath. BK was added in a volume of 250 µl from a stock solution of 10 µM for BK resulting in a final concentration of 0.5 µM in the perfusion chamber. This dose was determined as an intermediate dose in preliminary dose-response experiments (Fig. 1). After 2 minutes perfusion was started again in order to wash out BK from the organ bath.

Colonic segments were studied from 4 experimental groups (each n=6, one segment was harvested from one animal):

1. Vehicle pretreated control mice
2. Mice with DSS colitis

1 3. Mice with DSS colitis and administration of the mast cell inhibitor
2
3 doxantrazole (10^{-4} M) in the organ bath to investigate effects of mast
4
5 cell mediator release on afferent nerve discharge during colitis
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7

8 4. Mice with DSS colitis and administration of the cyclooxygenase
9
10 inhibitor naproxen (10^{-5} M) in the organ bath to investigate effects of
11
12 mediators from the cyclooxygenase pathway on afferent nerve
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14 discharge during colitis
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22 Preparations in the experimental groups 3. and 4. were superfused with Kreb's
23
24 (containing nifedipine 1 μ M) at baseline before the superfusate was replaced
25
26 by Kreb's containing nifedipine plus doxantrazole (10^{-4} M) or naproxen (10^{-5} M)
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28 for 15 minutes before distension was started. The protocol was then continued
29
30 as described above. Concentrations of doxantrazole and naproxen were
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32 chosen according to previous *in vitro* experiments (15, 16). A potential effect of
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34 doxantrazole and naproxen on afferent nerve discharge in naïve control
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36 animals was investigated in a separate series of experiments.
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47 *Assessment of colitis*

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50 Animals overall condition was assessed by quantifying loss of total ~~body~~
51
52 ~~weight~~body weight between treatment day one and eight. After harvesting the
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54 colon, presence of colitis was assessed morphologically by determination of
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56 colonic length which was measured from the beginning of the ascending colon
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(without cecum) to the peritoneal deflection of the upper rectum, and ratio between colonic weight and length indicating thickening of the colonic wall.

Colonic segments were also harvested from separate colitis and control animals that were used exclusively for histological assessment (each n=6). Segments were fixed in 4% paraformaldehyde and processed for histology. Slides were stained with haematoxylin and eosin. Inflammation was assessed by a previously published scoring system (Table 1, 17).

Drugs

DSS was purchased from MP Biomedicals (Aurora, OH, USA). Doxantrazole, naproxen, BK, and heavy liquid paraffin were obtained from Sigma Chemicals, Munich, Germany).

Data analysis

Multi-unit mesenteric afferent nerve recordings contained action potentials with different amplitudes and waveforms that were separated from noise and artefacts with the help of waveform imaging software (spike2, CED, as above). For this purpose, units in the multi-unit signal were identified with spike2, separated and those consisting of action potentials were put back together to a multi-unit signal that was subsequently quantified.

~~single-unit analysis by waveform imaging software which is based on different~~

1 amplitudes and waveforms of action potentials in the multi-unit recording. In-
2 brief, Spike2 software automatically sets templates for spikes in the recorded
3 signal and then counts spikes that match these ~~carefully defined~~ templates
4 selectively. Templates from signals that did not resemble action potentials
5 eliminated as artefacts. ~~This data is used to construct peri-stimulus histograms~~
6 of individual units within the whole nerve recording. Each template is
7 constructed from 65 data points. The parameters used when setting up
8 templates for single action potentials could be varied but typically we require at
9 least 60% of the data points to match the predefined template with an
10 error of < 2%. Details of this waveform based single unit analysis were
11 previously published (18).
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33 In general, multi-unit afferent discharge of the mesenteric nerve was evaluated
34 as total discharge above baseline. Specifically, afferent nerve firing to
35 distension was determined by quantifying the increase of peak firing frequency
36 above baseline over a three second period at 20 cmH₂O increments in
37 intraluminal pressure. The response to BK was expressed as peak increase of
38 impulses per second above baseline from a three seconds bin size. Baseline
39 discharge frequency was calculated as peak firing for two minutes prior to
40 distension of BK administration. Data are given as mean ± SEM and were
41 compared by one-way ANOVA and post-hoc Bonferroni correction. Differences
42 were considered statistically significant for $p < 0.05$.
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Results

Morphology and histology of DSS - colitis

At day 8 after the beginning of DSS treatment, mice had developed colitis that was characterized by diarrhea and visible fecal blood which was observed in all DSS-treated animals but never in controls. Colitis was accompanied by weight loss, shortening of the colon, and increased ratio of colonic weight / length (all $p < 0.001$; Table 2). Histological workup was performed in a representative series of six colitis and control animals. It revealed erosions, ulcerations, infiltration with polymorphonuclear cells, severe submucosal edema, and thickening of the bowel wall in DSS-treated mice (see Fig. 2 for representative images). The microscopic score determined by the scoring system given in Table 1 was 6.0 ± 0.2 in DSS mice compared to 1.0 ± 0.4 in controls ($p < 0.001$; $n=6$ each).

Mesenteric afferent nerve recordings

The recording signal was characterized by high amplitude spikes with varying amplitudes and waveforms. In addition, smaller spikes were observed during recordings that could not be separated from background noise. Thus, only high amplitude spikes were further quantified and analyzed as single units by waveform discriminator software as described above.

1 The average peak firing frequency at baseline was $11 \pm 2 \text{ imp sec}^{-1}$ in colitis
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3 segments which was higher than $5 \pm 1 \text{ imp sec}^{-1}$ in non-inflamed control
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5 segments ($p < 0.05$). In colitis segments used to study the effect of doxantrazole
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7 and naproxen, discharge was $10 \pm 1 \text{ imp sec}^{-1}$ at baseline following
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9 doxantrazole administration (10^{-4} M) and $8 \pm 1 \text{ imp sec}^{-1}$ following naproxen
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11 (10^{-5} M) which was not different from recordings from colitis segments without
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13 doxantrazole or naproxen.
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22 BK administration into the organ bath was followed by a robust increase in
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24 afferent nerve discharge. This increase in discharge was based on
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26 spontaneously active fibers that increased their discharge frequency and
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28 recruitment of fibers that were silent at baseline (Figure 3A). Peak increase in
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30 afferent nerve discharge above baseline to BK was $23 \pm 6 \text{ imp sec}^{-1}$ in control
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32 segments, while an increase of $47 \pm 7 \text{ imp sec}^{-1}$ was recorded in colitis
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34 segments ($p < 0.05$, Figure 3B). Afferent discharge to BK in colitis segments
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36 was $23 \pm 2 \text{ imp sec}^{-1}$ following doxantrazole (10^{-4} M) and $31 \pm 5 \text{ imp sec}^{-1}$
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38 following naproxen (10^{-5} M) which was not different from non-inflamed control
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40 segments.
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53 During continuous ramp distension, a pressure dependent increase in afferent
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55 nerve discharge above baseline was observed (Figure 4A). Mechanical
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57 sensitivity in colitis segments was increased compared with non-inflamed
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control segments over the whole range of distending pressures from 0 to 80 cmH₂O (p<0.05, Figure 4B). Peak discharge of all analyzed units increased from 14 ± 2 imp sec⁻¹ in non-inflamed control tissue to 24 ± 5 imp sec⁻¹ in colitis segments at 80 cmH₂O (p<0.05). Treatment with doxantrazole (10⁻⁴ M) or naproxen (10⁻⁵ M) reduced the increase of peak afferent discharge at 80 cmH₂O in colitis segments to 13 ± 1 imp sec⁻¹ and 14 ± 3 imp sec⁻¹ which was not different from non-inflamed controls.

Doxantrazole and naproxen were additionally given in naïve control experiments and shown to have no effect on mesenteric afferent nerve discharge in animals without colitis (Figure 5).

Discussion

This study investigates the role of mast cells and the cyclooxygenase pathway for intestinal afferent sensitivity during DSS colitis which is an established model of inflammatory bowel disease (12, 13). Mesenteric afferent nerve discharge at baseline and to BK and mechanical ramp distension was increased during DSS colitis compared to control tissue. Afferent sensitivity to both stimuli returned to control levels when colitis segments were pre-treated with either the mast cell stabilizer doxantrazole or the non-selective cyclooxygenase (COX) inhibitor naproxen in the organ bath. A similar presence of inflammation in all colitis subgroups was confirmed by macroscopic and histological assessment with an established scoring system.

Baseline discharge was higher in colitis animals compared to normal controls which was uninfluenced by doxantrazole or naproxen. This increase in discharge during colitis is likely to be attributed to an array of mediators released during intestinal inflammation such as cytokines, biogenic amines, prostanoids, leucotrienes, nucleosides etc. that have all the potential to sensitize afferents (19, 20). As doxantrazole or naproxen only inhibit subgroups of these mediators, uninhibited agents are likely to be sufficient to maintain sensitization with subsequently increased discharge at baseline. Alternatively, differences in afferent discharge in the presence of these antagonists may simply be not big enough to be unveiled at baseline in

1 contrast to more pronounced responses that are triggered when additional
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3 stimuli are administered.
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9 We established a technically challenging novel *in vitro* preparation to record
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11 from afferent nerve fibers from the mesentery of the proximal colon. Previous
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13 reports described multi-unit afferent nerve recordings remote from the colon i.e.
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15 from the pelvic or splanchnic nerve (6, 8). The advantage of this novel setup is
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17 that afferent nerve signaling was recorded directly and close to the site of its
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19 generation, minimizing potential modulatory neural input and allowing a
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21 precise localization of the corresponding area of the colon which gave rise to
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23 these afferent nerve signals. ~~where afferent nerve signals originated from.~~
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34 As for other experimental setups serving to record from colonic afferents *in*
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36 *vitro* (14), it was indispensable to add the L-type Ca^{2+} -channel blocker
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38 nifedipine to the organ bath because otherwise spontaneous colonic motor
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40 activity triggers artificial increases in afferent nerve discharge that render
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42 interpretation of afferent nerve responses to specific stimuli impossible.
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46
47 Abundant previous work documents that nifedipine does not eliminate afferent
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49 discharge to an array of inflammatory mediators sensitizing intestinal afferents
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51 (18, 21, 22). However, mesenteric afferent firing to more complex stimuli such
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53 as intestinal ischemia may be reduced by nifedipine, potentially by modulation
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55 L-type Ca^{2+} -channels which may inhibit mediator release from immune cells
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(23, 24). Thus, we cannot rule out that nifedipine may have had an attenuating effect during colitis, however, as all groups received this agent in an identical fashion, modulation of afferent nerve responses by doxantrazole and naproxen can nevertheless be attributed to these agents.

Serosal superfusion of the preparation with BK elicited a robust afferent nerve response which was increased in colitis animals compared to controls. BK is released during inflammation and secondary to tissue damage (25) acting subsequently as a key mediator for the generation of pain (11). It binds to two G protein-coupled receptors which were named B₁ and B₂ (26). The B₂ receptor is constitutively expressed and mediates afferent nerve sensitivity in naive animals (26, 27). Increased afferent sensitivity to BK during colitis suggests that other inflammatory mediators may have sensitized afferent nerves to BK. These are likely to originate from mast cells as mast cell stabilization by pretreatment with doxantrazole resulted in an afferent nerve response which was similar to naïve animals. Among these mast cell mediators, prostaglandin E₂ (PGE₂) is a likely candidate as expression of Cox-2 is upregulated during DSS colitis and PGE₂ was previously shown to sensitize the mesenteric afferent nerve response to BK in naive animals (27). Indeed, inhibition of prostaglandins with naproxen also reduced the afferent nerve response back to control levels as observed during doxantrazole pretreatment. Furthermore, prostaglandins are known to be released during

1 inflammatory responses that are predominantly brought on by mast cells (19,
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3 20).
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9 Sensitization of the afferent nerve response was also observed during
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11 continuous intraluminal ramp distension. This way to test mechanosensitivity
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13 excites different populations of afferent nerve fibers i.e. parasympathetic and
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15 sympathetic afferents plus centrifugal fibers projecting to the prevertebral
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17 ganglia (28). The latter population is small and its contribution to the afferent
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19 nerve response was previously shown to be neglectable (29), while the bulk of
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21 afferent firing consists of low-threshold parasympathetic afferents and
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23 wide-dynamic range and high-threshold afferents of sympathetic origin (29, 30,
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25 31). Thus, afferent nerve discharge at low distension pressures stems
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27 predominantly from parasympathetic afferents and at high distension
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29 pressures (representing a noxious event) from sympathetic afferents (30, 31).
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39 In the present study, the afferent nerve response was sensitized over the
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41 whole pressure range in colitis animals compared to controls. This suggests
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43 that both types of afferent nerve endings were sensitized, i. e. parasympathetic
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45 and sympathetic afferent nerve endings.
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53 Increased responses during mechanical ramp distension may be explained by
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55 a permissive action of other proinflammatory mediators that were released
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57 during DSS colitis. Among these are several mast cell mediators such as 5-HT
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1 and histamine which are known to sensitize parasympathetic nerve endings
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3 (14, 18, 21), and prostaglandins and kinins (27) which are known to sensitize
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5 predominantly sympathetic afferents. Furthermore, some mediators such as
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7 adenosine exist that have the potential to sensitize both parasympathetic and
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9 sympathetic afferents (32, 33). As for bradykinin, afferent nerve responses to
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11 mechanical ramp distension were reduced by both, the mast cell stabilizer
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13 doxantrazole and the cyclooxygenase inhibitor naproxen. There are several
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15 possible explanations for this phenomenon that both drugs worked. One is that
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17 DSS colitis triggers the release of a “soup” of inflammatory mediators that all
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19 sensitize afferents in a joint action. Thus, reducing different components by
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21 different inhibitors in such a “soup” may have a similar effects on afferent nerve
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23 discharge to bradykinin and mechanical stimulation. Another likely explanation
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25 is that mast cell action on afferent nerve terminals works predominantly via
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27 prostanoids from mast cells. This would also explain why both,
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29 pharmacological mast cell stabilization and inhibition of prostanoid release
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31 have a similar effect on afferent nerve discharge.
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47 In conclusion DSS colitis is accompanied by inflammation that is characterized
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49 by morphological alterations of the proximal colon. Afferent sensitivity to BK
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51 and to mechanical stimuli of different intensity is increased during DSS colitis
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53 suggesting that parasympathetic and sympathetic afferent nerve fibers are
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55 sensitized during DSS induced colitis. The underlying mechanisms seem to
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1 involve mast cells and the cyclooxygenase pathway, so that mast cell
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3 stabilization and/or COX inhibition represent potential strategies to reduce
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5 afferent sensitization and subsequent pain during colitis.
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Legends

Figure 1

Dose-response relationship for mesenteric afferent nerve discharge to rising doses of serosal bradykinin. Note that the 0.5 μ M dose is neither a minimal nor a submaximal dose as regards the response in afferent nerve discharge. d/c: discharge; white bar: 0.25 μ M, bar with horizontal stripes: 0.5 μ M, black bar: 1 μ M. Data are mean \pm SEM (n=6).

Figure 2

Hematoxilin and Eosin staining of colon from a DSS pretreated animal with colitis (left) and a control animal that received vehicle (right). During DSS colitis, crypts were absent in large areas, wall thickness was increased, and cellular infiltrates were observed in all layers of the gut wall. Scale bars are 0.5 cm.

Figure 3

Panel A shows a r Representative recording and analysis of an afferent nerve response to bradykinin in a control animal (BK; 0.5 μ M). BK administration into the organ bath was followed by a robust increase in afferent nerve discharge which is shown by the raw nerve signal (top trace). Sequential rate histograms below depict the number of impulses during the multi-unit afferent nerve response . _

Panel B displays a corresponding recording from a colitis animal that was pretreated with DSS.

Panel [CB](#) summarizes the afferent nerve discharge data which are given as peak discharge above baseline (white bar: controls, black bar: colitis; horizontal stripes: colitis plus doxantrazole (10^{-4} M); oblique stripes: colitis plus naproxen (10^{-5} M)). Note that both doxantrazole and naproxen reduced afferent nerve discharge to BK during intestinal inflammation when compared to vehicle controls (all n=6, mean \pm SEM; *p<0.05 versus control, #p<0.05 versus colitis).

Figure 4

Recordings of afferent nerve discharge following intraluminal ramp distension serving as a mechanical stimulus. A: Representative recording from a control experiment. The upper trace shows the raw nerve recording, while the middle trace displays the multi-unit afferent nerve response in sequential rate histograms of mesenteric afferent nerve discharge frequency. Pressures during ramp distension are given in the bottom trace. [B: Corresponding recording from a colitis animal that was pretreated with DSS.](#)

[CB](#): This diagram shows summarized data of peak afferent nerve discharge above baseline in each experimental group. Note that pre-treatment with doxantrazole (10^{-4} M) and Naproxen (10^{-5} M) in the organ bath prevented an increase in afferent nerve discharge during intestinal inflammation when compared to vehicle controls (all n=6, mean \pm SEM; *p<0.05 versus control, #p<0.05 versus inflammation).

Figure 5

Afferent discharge from mesenteric colonic afferents in naïve animals (no DSS-colitis) during vehicle (white bars), naproxen (oblique bars; 10^{-5} M) or doxantrazole (10^{-4} M; horizontal striped bars). Panel A shows afferent discharge to bradykinin ($0.5 \mu\text{M}$) which is not different in the three subgroups (n.s.; mean \pm SEM; n=6). Panel B shows afferent discharge during continuous intraluminal ramp distension at different luminal pressure levels. No difference was observed following pretreatment with naproxen or doxantrazole compared to vehicle controls (n.s.; mean \pm SEM; n=6).

Table 1

Scoring system for histological assessment of inflammation (see reference 14)

Epithelium		
Normal morphology		0
Loss of goblet cells		1
Loss of goblet cells in large areas		2
Loss of crypts		3
Loss of crypts in large areas		4
Infiltration		
No infiltrates		0
Infiltrate around crypt basis		1
Infiltrate reaching to <i>L. muscularis mucosae</i>		2
Extensive infiltration reaching the		
<i>L. muscularis mucosae</i> and thickening of		
mucosa with severe oedema		3
Infiltration of the <i>L. submucosa</i>		4

Table 2

	Body weight change (g)	Colonic length (cm)	Colonic weight/length ratio (mg cm ⁻¹)
Controls	2.0 ± 0.4	5.9 ± 0.2	30.5 ± 0.6
DSS colitis	- 4.4 ± 0.4*	3.3 ± 0.2*	38.6 ± 1.3*
DSS colitis plus pre-treatment with doxantrazole <i>in vitro</i>	- 4.2 ± 0.5*	3.6 ± 0.1*	38.5 ± 2.0*
DSS colitis plus pre-treatment with naproxen <i>in vitro</i>	- 4.5 ± 0.3*	3.4 ± 0.2*	38.0 ± 1.4*

Data characterizing DSS colitis induced by adding DSS (3 %) in the animals' drinking water for 7 days. Control mice received normal tap water. Mice had colitis at day 8 after the beginning of DSS treatment characterized by weight loss, colonic shortening, and thickening of the colonic wall expressed as weight / length ratio. Data are mean ± SEM, *p<0.001 compared to controls (ANOVA).

Figure 1

Figure 1

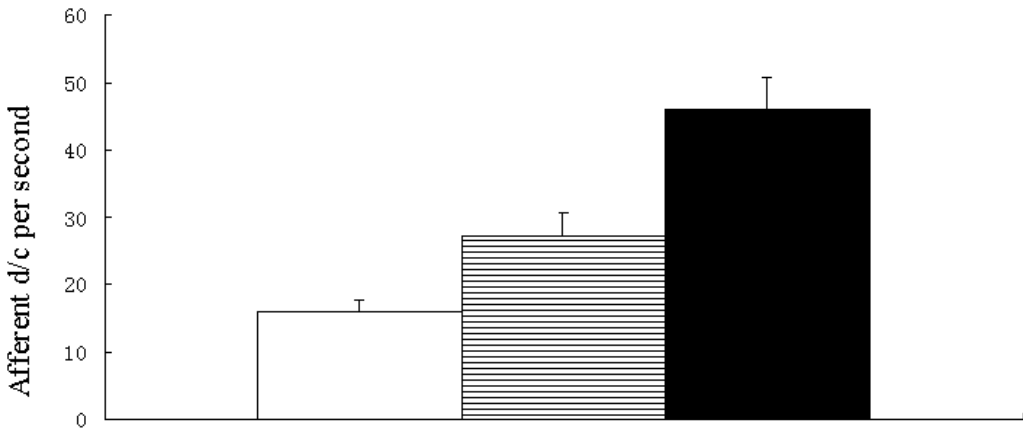


Figure 2

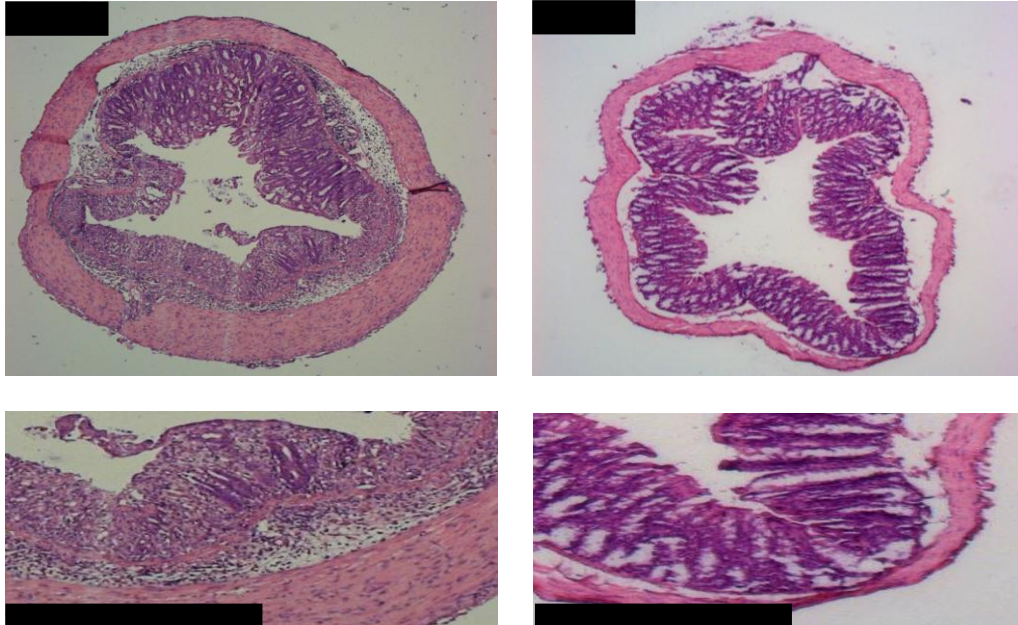
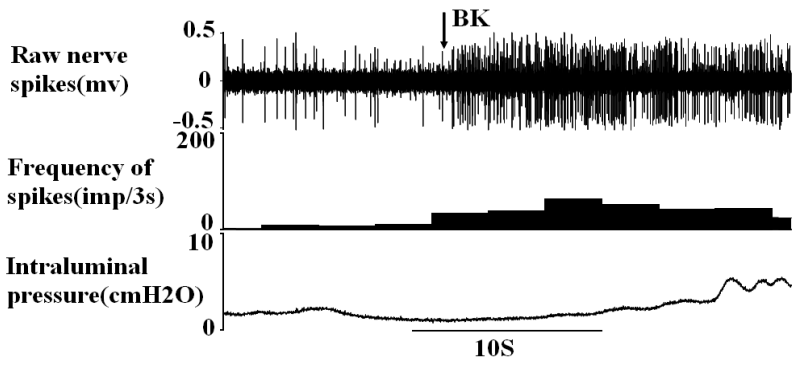


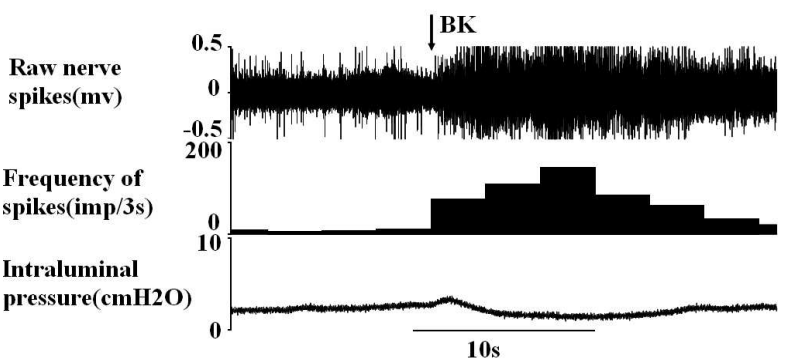
Figure 3

Figure 3

A



B



C

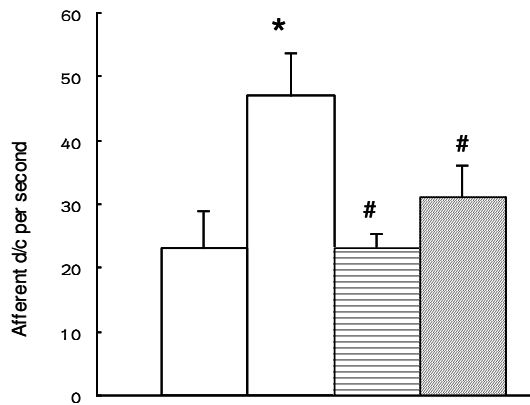
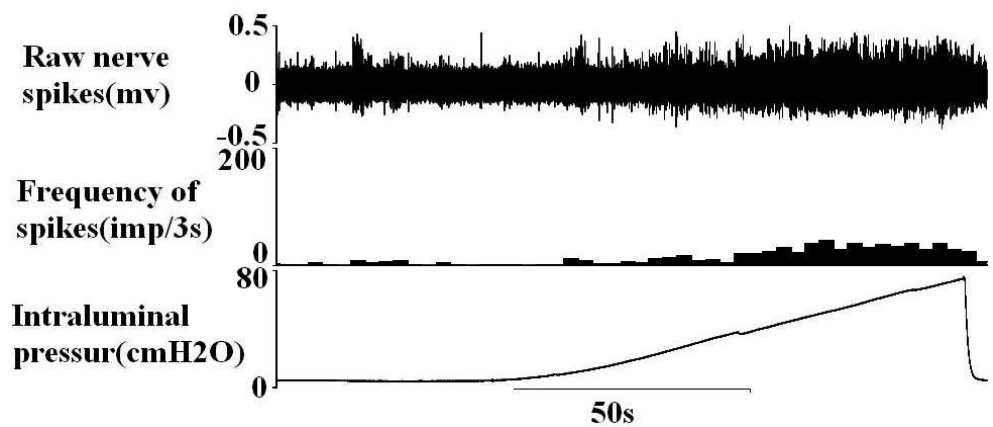


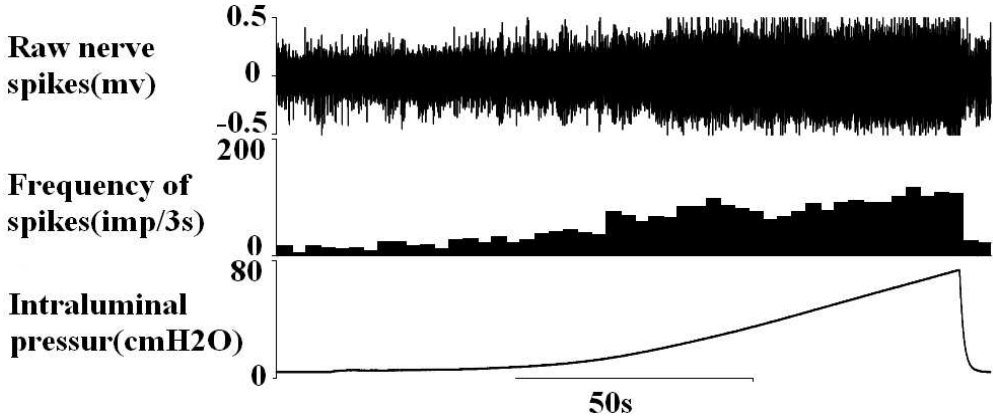
Figure 4

Figure 4

A



B



C

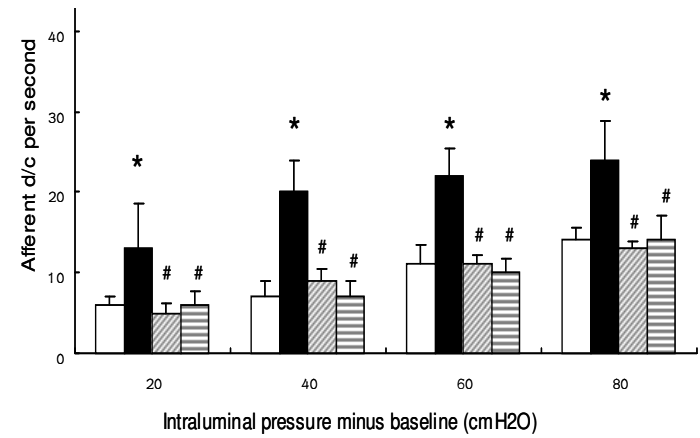
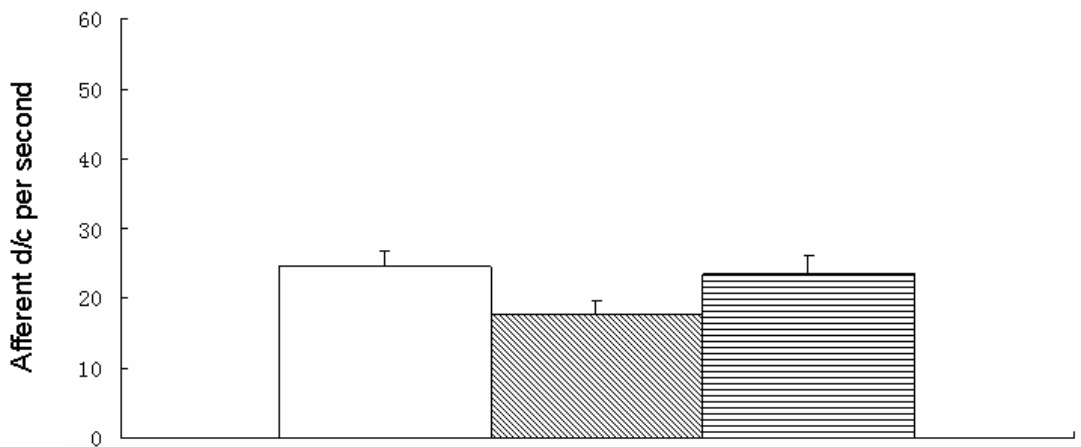


Figure 5

Figure 5

A



Response to bradykinin

B

